

A METHOD AND COMPOSITION TO ELICIT AN EFFECTIVE AUTOLOGOUS
ANTITUMORAL IMMUNE RESPONSE IN A PATIENT

1 BACKGROUND OF THE INVENTION

2

3 Claim of Priority

4 The present application is based on and a claim to priority is
5 made under 35 U.S.C. Section 119(e) to the provisional patent
6 application currently pending in the U.S. Patent and Trademark
7 Office having Serial No. 60/391,674 and a filing date of June 26,
8 2002.

9

10 Field of the Invention

11 The present invention relates in general to a method and a
12 composition to elicit an effective antitumoral immune response in
13 a patient, specific to his or her own tumor antigens (i.e. an
14 autologous antitumoral immune response). More specifically, the
15 present invention relates to a method to elicit an effective
16 autologous antitumoral immune response in a cancer patient which
17 comprises generating, preserving, and storing specific tumor
18 associated antigens, and eliciting the autologous antitumoral
19 immune response, at least in part, through a combination of dual
20 vaccines. The present invention further provides for enhancement
21 of the antitumoral immune response resulting from an internal
22 vaccine and an external vaccine by activating antigen presenting
23 cells, as well as by inhibiting a tolerance immune response in

1 cancer patients. The present invention further provides a method
2 for preparing an autologous hemoderivative composition for
3 utilization in the inventive method as an external vaccine.

4

5 DESCRIPTION OF THE RELATED ART

6 Initially, immunotherapy techniques began with treatments or
7 vaccines for the prevention of infectious diseases, and they have
8 since virtually eradicated many such diseases. Generally,
9 immunotherapy has exploited the ability of an immune system to
10 respond when it is in contact with alien molecules known as
11 antigens. An immune response is specifically addressed against
12 antigenic molecules or against other organisms that express these
13 antigenic properties, collectively known as antigens. When the
14 antigen is not a living organism, the immune response is frequently
15 mediated by cells identified as helper cells or CD4+ lymphocytes,
16 and by antibody producing cells, the final effectors being
17 antibodies, for example, immunoglobulin molecules that are
18 circulating throughout a patient's bloodstream. When the antigen
19 is a component of a live cell or microorganism, the immune response
20 is mediated by circulating cells and also the effector cells,
21 mainly, cytotoxic CD8+ lymphocytes.

22 Frequently, the presence of antibodies against a specific
23 antigen can be tested by the immediate (20-60 minute) response
24 obtained when a dermic test with the antigen is performed. The
25 cellular response is tested by a delay (48-72 hour) response after

1 dermic exposure to the antigen. As a consequence, the antibodies
2 and the cellular immune response to them are also known as
3 immediate response or delay response, respectively.

4 Most of the time, the immune response is associated with
5 cooperation between the antibodies, or the molecular mediated arm,
6 and the cellular mediated arm. Typically, the cooperative response
7 is the antibodies known dependent cytotoxic response. Classical
8 immunotherapy techniques have used such antigens as vaccine agents.
9 These agents were treated to avoid their pathogenicity and/or they
10 were mixed with adjuvants in order to facilitate their
11 accessibility, recognizance or stimulant activity. Antigens are
12 necessary for immune response because, by definition, an immune
13 response is a specific antigen-addressed response, however, modern
14 research has recognized that sometimes although antigens are
15 present, their immunological power is not enough to stimulate an
16 effective immune response. In such cases, the immune response can
17 be elicited by other substances or by modified antigens with more
18 powerful antigenic activity and cross-reactivity with the specific
19 target of the immune response. In addition, some agents have been
20 identified which elicit immune responses not upon specific
21 antigens, but, rather, upon specific or global reactive portions of
22 the immune system. As a consequence, today it is more appropriate
23 to identify this whole family of compounds which may be used in
24 immunotherapy, including specific antigens and all other agents
25 that elicit or enhance a response against antigens or from the

1 immune system, collectively, as immunogens.

2 It is appreciated that human cancer immunotherapy has been in
3 use and has been subject of reported research for years. More in
4 particular, human cancer immunotherapy began when specific antigens
5 in malignant cells were recognized. With this knowledge, the
6 stimulation of a patient's immune response against the specific
7 antigens of these malignant cells as an antitumoral treatment was
8 explored. Along with surgery, chemotherapy, and radiotherapy,
9 immunotherapy provides yet another therapeutic technique available
10 in Oncology. Frequently, these therapeutic techniques are employed
11 simultaneously or successively in various treatment regimens.

12 Cancer immunotherapy techniques are commonly grouped into one
13 of two categories, namely, non-specific immunotherapy or specific
14 immunotherapy. The goal of non-specific cancer immunotherapy is an
15 increase in all of a patient's immune responses, thereby improving
16 the activity level throughout the patient's immune system.
17 Specific cancer immunotherapy, on the other hand, has the goal of
18 stimulating a singular antitumoral immune response that may be
19 directed against the patient's tumor or the patient's tumor type or
20 an antigen of that tumor.

21 Each of these immunotherapy techniques may be further grouped
22 into sub-categories, being either an active immunotherapy or an
23 adoptive immunotherapy. Active immunotherapy techniques comprise
24 methods wherein the immune response induced by treatment is
25 dictated by the patient's own immune system, whereas, adoptive

1 immunotherapy techniques comprise methods in which one, several, or
2 all of the components of the patient's immune system are replaced,
3 thereby dictating an alternate immune response.

4 Thus, there are in actuality, four commonly known
5 immunotherapy techniques utilized in the field of cancer treatment,
6 specifically: active non-specific immunotherapy; adoptive
7 non-specific immunotherapy; active specific immunotherapy; and,
8 adoptive specific immunotherapy. Numerous agents have been
9 produced, modified, or protocolized by different methods in order
10 to be employed as immunogens in one or more of these four cancer
11 immunotherapy techniques. Each these techniques, however, exhibit
12 certain shortcomings which hinder their development and limit their
13 implementation as effective and safe cancer treatment regimens,
14 except for short periods of time and only for a small number of
15 select tumor types. A description of each of these four cancer
16 immunotherapy techniques is presented below in further detail,
17 including the known shortcomings of each.

18 Active non-specific immunotherapy includes the administration
19 of a biological or chemical agent that has been proven to stimulate
20 immune system activity. Compositions comprising bacille Calmette-
21 Guerin(BCG), *Corynebacterium*, levamisole, and zinc compounds have
22 been among the most tested immunogens. The basic supposition is
23 that cancer patients are always immune-depressive and that this
24 technique could restore the immune system activity, including
25 antitumoral response. It must be noted, however, that the supposed

1 global depression of the immune system is yet to be demonstrated in
2 most cancer patients, thus, the global immune-restoration is not
3 necessarily a proper goal of treatment, and the possible secondary
4 and potentially negative effects of non-specific immunotherapy are,
5 therefore, not justified. In fact, in some cases it has been
6 reported that non-specific hyper-stimulation of the immune system
7 has also produced enhancement or tumor progression. This result
8 may be explained by the stimulation of cell populations with
9 properties of tolerance or suppression.

10 Adoptive non-specific immunotherapy comprises one variation
11 involving the transfer of immunocompetent cell precursors, known as
12 source cells, from a donor to a receptor in order to allow their
13 proliferation, thereby resulting in the quimeric regeneration of
14 immune system cell populations. In particular, tests have been
15 performed on the transfer of a defined sub-population of
16 immunocompetent cells to determine if it may increase their
17 function. A common and well known treatment regimen utilizing this
18 technique is an allogenic bone marrow transplant. One of the main
19 drawbacks of this technique is the prevalence of reactions (i.e.
20 rejection) of the transplanted bone marrow by the host.

21 More recently, another variation of adoptive non-specific
22 immunotherapy has been tested which employs the administration of
23 select components of the immune system which are candidates to
24 promote a more amplified immune response. For example, recombinant
25 molecules that are normally mediators of immune response, such as

1 interferons and interleukins, have been the agents produced by
2 genetic recombination and employed as immunogens in order to expand
3 antitumoral responses. These compounds are known as biological
4 response modifiers and they are active as antitumoral agents but
5 only in a few specific types of tumors such as, renal cell
6 carcinoma, melanoma, hairy cell leukemia, and non-Hodgkin's
7 lymphoma. Additionally, even when used to treat these specific
8 types of tumors, most of the benefits are partial and temporary.
9 The problem with this technique appears to be that the
10 rate-limiting step of antitumoral immune response and the target
11 step of a biological response modifier acting in isolation are
12 presently unknown. As a consequence, the effectiveness of any
13 treatment with these agents is fortuitous, at best.

14 Adoptive specific immunotherapy is a method of treatment that
15 uses lymphocytes which have previously been in contact with tumor
16 cell antigens, either in vivo or in vitro. In addition, this
17 immunotherapy technique uses recombinant monoclonal antibodies
18 against specific molecular targets expressed by malignant tumor
19 cells. The antecedents of this procedure are the treatment of
20 infectious diseases with hyperimmune serum or immunoglobulins.
21 Subsequently, in the field of cellular mediated immunity, the
22 intent was to collect tumor infiltrating lymphocytes or dendritic
23 cells and to re-inject them with previously known pulse activation
24 or clonal expansion.

25 Currently, there is active development directed towards the

1 use of recombinant monoclonal antibodies directed against molecular
2 tumor targets which represents a variation of adoptive specific
3 immunotherapy. Components of tumor receptors such as H2-neu and
4 CD20, which may be over-expressed in cells of some breast cancers
5 and non-Hodgkin's lymphomas, respectively, are the most effective
6 target for the monoclonal antibodies currently available for
7 immunotherapy. The effectiveness of the treatment of patients
8 having malignant diseases with monoclonal antibodies also appears
9 to be more the exception than the rule. The remissions are
10 frequently limited to only a fraction of patients treated and
11 having tumors with the supposed antigenic target, and these
12 remissions are generally only temporary.

13 The difficulties encountered with this immunotherapy technique
14 appear to be that molecular changes or losses in the target of the
15 transferred immune effector are very frequent due to malignant
16 disease evolution. This is due, at least in part, because tumor
17 cells exhibit a high rate of spontaneous mutation as a consequence
18 of their high proliferative turnover and their high rate of
19 mutation induced by oncological therapies. This results in an
20 immunological escape mechanism which detracts from the
21 effectiveness of this immunotherapy technique.

22 Lastly, active specific immunotherapy utilizes a vaccine
23 comprising tumor specific antigens, known as neo-antigens or tumor
24 associated antigens (TAA). The existence of TAA has been well
25 recognized for some time. This immunotherapy technique includes

1 all vaccine treatments that include the administration of antigens
2 as tumor cells, tumor extracts, or as purified molecular compounds
3 extracted from tumors. In order to enhance the elicited immune
4 response, different procedures have been tested including alternate
5 methods of inoculation (e.g. intradermal, subcutaneous,
6 intramuscular, or intravenous), as well as the use of different
7 adjuvants (e.g. tumor cells with genetic engineering to secrete
8 immune-modulating cytokines, antigen pulsed dendritic cells, mixed
9 antigens with BCG, tumor peptide antigens combined with chaperone
10 heat shock proteins, and hapten potentiation of antigens).

11 In the last decade, active specific immunotherapy techniques
12 have been developed utilizing autologous systems, the goal being to
13 obtain a more specific immune response against well-demonstrated
14 tumor cell antigens specifically expressed by an individual tumor.
15 This represents a significant advance in active specific
16 immunotherapy because it allows customization of the immunotherapy
17 to an individual antigen profile that is generated in a specific
18 tumor by spontaneous and therapeutically associated gene tumor cell
19 mutations, through the individual patient-tumor history. Clinical
20 assessment of such active specific immunotherapy techniques,
21 however, indicates that it has only produced effective results in
22 the treatment of a few tumors and, once again, the results obtained
23 are only partial and are only temporary.

24 The difficulties encountered in active specific immunotherapy
25 techniques are mainly twofold. The first problem is related to the

1 basic nature of cancer itself. More in particular, the malignant
2 cells derived from a normal patient are the patient's self-cells
3 and, therefore, their molecular composition is not normally
4 antigenic relative to the host (i.e. the patient's) immune system.
5 The molecules in tumor cells that are unrecognizable as the
6 patient's self-cells are products of etiological or therapeutical
7 mutations and/or specific epigenetic structural modifications. The
8 concentration of these antigenic compounds are typically low in
9 most malignant tissues, and their antigenicity is further reduced
10 because the antigens are normally stored within the malignant
11 cells, far from the afferent immune system. Additionally, these
12 stored antigens are frequently destroyed by proteolysis when the
13 malignant tumor cells die by programmed death or apoptosis, unless
14 they are first protected, such as by protein induced cell stress.

15 The second difficulty encountered in active specific
16 immunotherapy relates to the preparation of a vaccine having the
17 patient's malignant tumor as its source. Here, both quantitative
18 and qualitative limitations are present. To begin with, the number
19 of inoculations and the amount of immunogen, or vaccine, in each
20 inoculation as required by this technique are limited by the
21 availability of surgical tumor specimens, and the typically weak
22 antigens which are present at low cellular concentrations therein.
23 In addition, and as noted above with respect to adoptive specific
24 immunotherapy techniques, if tumor cells modify their antigenic
25 profile due to their high rate of mutation, the immune effectors

1 elicited by inoculation of the original vaccine may not recognize
2 a target in the remaining mutated tumor cells. As a result,
3 repeated inoculations of the original vaccine will not usually be
4 effective unless current surgical tumor specimens are available in
5 order to prepare vaccines containing the successively mutated
6 antigens, however, such current surgical tumor specimens are
7 hardly, if ever, available.

8 Thus, it would be beneficial to provide a method to elicit an
9 antitumoral immune response in a cancer patient and, more in
10 particular, an effective autologous antitumoral immune response
11 thereby providing a new, improved, and innovative active specific
12 immunotherapy technique. Additionally, it would be helpful to
13 provide a method to elicit such an effective autologous antitumoral
14 immune response in a cancer patient via a treatment regimen
15 structured to modify an antigen library of tumor cells, or TAA,
16 thereby increasing the antigenicity relative to the patient's
17 immune system. It would also be desirable for such an improved
18 method to utilize a dual vaccine regimen including both an internal
19 vaccine comprising the endogenous release of TAA from the tumor
20 itself, as well as an external vaccine comprising a composition
21 derived from an autologous blood specimen obtained from the patient
22 at a plurality of discreet time periods over the course of the
23 entire treatment regimen. Any such method would further benefit
24 from the provision of a procedure to enhance the antitumoral immune
25 response in a patient via the activation of an antigen presenting

1 cell or APC population, and to inhibit a tolerance immune response
2 in the patient. In addition, a method for preparing a
3 hemoderivative composition from an autologous blood specimen for
4 use as an external vaccine would be desirable.

5

6 SUMMARY OF THE INVENTION

7 In view of the many drawbacks inherent in the immunotherapy
8 techniques currently known and used in the treatment of cancer, and
9 as otherwise identified in the art, the present invention provides
10 a method and a composition to elicit an antitumoral immune response
11 in patients, thereby providing a new improved active specific
12 technique for practicing cancer immunotherapy. It is noted that
13 the method of the present invention comprises certain aspects of
14 procedures known in medical practice and/or in medical research,
15 such as treatment with cytokines, colony stimulating factors
16 frequently used for hematological and immunological restoration,
17 application of chemotherapy and indomethacin, which have been used
18 and are the subject of continued research as antitumoral
19 treatments, and the malignant cell autoschizis promoted by
20 high-doses of ascorbic acid and menadione. Numerous other
21 procedures employed by the present invention, however, are not
22 known in the art, such as, by way of example only, an enhanced
23 generation and subsequent in vivo storage of specific TAA in the
24 tumor cells of a cancer patient, and a dual vaccine regimen
25 including an internal vaccine comprising a release of previously

1 generated, preserved, and stored endogenous TAA from the tumor
2 cells of the patient, and an external vaccine comprising an
3 autologous hemoderivative composition. The procedures utilized by
4 the method of the present invention, whether previously known or
5 initially presented herein, utilize various compounds which are
6 known in human pharmacology and approved for medical practice all
7 over the world. The innovation of the present invention lies in
8 the way these known compounds and procedures are utilized in
9 combination with the inventive procedures presented herein to
10 achieve the objectives of the present invention.

11 It is hereby asserted that the present invention defines an
12 inventive method which allows the practice of a new, improved, and
13 innovative autologous active specific immunotherapy technique. More
14 importantly, the method of autologous active specific immunotherapy
15 defined by the present invention is distinguishable from all
16 previously known immunotherapy techniques, such as those described
17 above. As an initial matter, it is noted that the method of the
18 present invention provides for an enhancement of tumor antigenicity
19 relative to an immune system of a cancer patient as a
20 distinguishing factor in autologous active specific immunotherapy.
21 Another distinguishing factor is that the inventive method
22 comprises a dual vaccine, one being an internal vaccine and another
23 being an external vaccine. As previously indicated, one vaccine is
24 an internal vaccine because after the storage of one or more
25 immunogens in the patient's tumor cells, the patient is

1 "vaccinated" by triggering the subsequent release of the immunogens
2 (e.g. antigens, TAA, or vaccine) from the tumor cells to the
3 interstitial spaces in the patient's body such as phagocytes,
4 lymphatic vessels and/or blood vessels. The other vaccine is an
5 external vaccine because the patient is vaccinated via a
6 subcutaneous inoculation, or other inoculation technique, with a
7 hemoderivative composition prepared from an autologous blood
8 specimen containing the one or more immunogens (e.g. antigen, TAA,
9 or vaccine).

10 As stated above, known autologous active specific
11 immunotherapy techniques for cancer utilize a surgical specimen of
12 a patient's tumor as a source of vaccine, which is another
13 important distinction of the present invention. In particular, the
14 known immunotherapy techniques which require surgical specimens of
15 the patient's tumor inherently comprise as a limiting condition the
16 availability of such a surgical specimen, which is rarely available
17 more than once, and even then, it is more than likely during the
18 initial stages of diagnosis and treatment. Therefore, it is not
19 possible to update the vaccine when utilizing known immunotherapy
20 techniques, as may be required if the remaining tumor changes its
21 antigenic expression, which is not an uncommon occurrence given the
22 high rate of mutation in such organisms.

23 Conversely, the present invention as described herein
24 eliminates the need for surgical specimens of the patient's tumor,
25 and rather utilizes the remnant tumor cells, and, more

1 specifically, the neo-antigens and/or TAA released from the remnant
2 tumor cells into the patient's bloodstream which provide the source
3 of immunogens for both an initial internal vaccine, as well as for
4 subsequent internal and external vaccines. As a result, the
5 present invention provides for a plurality of vaccines which may be
6 repeatedly updated so as to be effective against the specific tumor
7 antigens as they change. This is possible because the present
8 invention utilizes the antigen library of the patient's remaining
9 tumor to provide the immunogen which is the target of the immune
10 response elicited from the internal vaccine and is subsequently the
11 source of the antigenic immunogen in the blood utilized to produce
12 an external vaccine. As such, the immunogen, and thus, the
13 internal and external vaccines, are always contemporary each time
14 the inventive method is employed.

15 As may be seen from the foregoing, the present invention
16 comprises a new, improved, and inventive method of autologous
17 active specific immunotherapy which, while incorporating certain
18 aspects of known cancer immunotherapy techniques, comprises
19 numerous novel features which eliminate many of the shortcomings of
20 these previously known techniques. Furthermore, none of the novel
21 features of the method of the present invention are anticipated,
22 rendered obvious, suggested, or even implied by any known
23 immunotherapy technique or other cancer treatment described herein
24 or otherwise known.

25 Turning now to a further description of the method of the

1 present invention, it is generally directed towards eliciting an
2 effective autologous antitumoral immune response in a cancer
3 patient and comprises generating a plurality of neo-antigens or
4 tumor associated antigens (TAA) in a plurality of tumor cells of
5 the patient, preserving the plurality of TAA in the plurality of
6 tumor cells, activating a plurality of antigen presenting cells
7 (APC), breaking or inhibiting an immune tolerance response,
8 triggering an internal vaccine in the patient, and providing the
9 patient an external vaccine comprising an autologous hemoderivative
10 composition. Additionally, the present invention comprises a
11 method for the preparation of an autologous hemoderivative
12 composition such as may be utilized in the foregoing method for
13 eliciting an effective autologous antitumoral immune response, as
14 well as the autologous hemoderivative composition. Additionally,
15 the present invention comprises a method for performing an
16 immunological assessment of an elicited immune response, as well as
17 for performing a clinical assessment of an elicited antitumoral
18 response.

19 To accomplish the objectives of the present invention, the
20 method includes generating a plurality of neo-antigens or tumor
21 associated antigens (TAA) in a plurality of tumor cells of the
22 patient. The plurality of TAA may include peptides and/or proteins
23 with molecular sites unrecognized as molecular components of the
24 patient's self-cells and, therefore, of the normal organic
25 composition, by the patient's immune system. These alien molecules

1 (i.e. TAA) are generated in malignant tumors, by genome
2 abnormalities or mutations. The mutated genes can be attributed to
3 etiopathogenesis of cancer (oncogenes, antioncogenes),
4 physiopathology of cancer (high proliferation fraction with high
5 rate of spontaneous mutations), or therapeutical interventions
6 (radio- and chemo- induced mutations). Mutated genes can generate
7 a plurality of TAA by their direct expression or by the promotion
8 of intracellular conditions eliciting epigenetic normal protein
9 transformation. In order to generate a plurality of TAA in tumor
10 cells, it is necessary to increase in these cells their protein
11 synthesis and mutation frequency.

12 Thus, the method also comprises inducing protein synthesis in
13 a plurality of tumor cells by treating the patient with a suitable
14 pharmaceutical compound in order to activate the growth
15 factor-receptors, such as are typically highly expressed in most
16 malignant cells. One pharmaceutical compound which is suitable for
17 this purpose is insulin, due to the insulin-like growth factor-
18 receptors which are highly expressed in many malignant cells.

19 Insulin action requires the agonism of a cellular
20 insulin-receptor. As result of this agonism, the receptor is
21 activated and several biological processes are started. Among the
22 processes activated by insulin-receptor agonism are protein
23 synthesis linked to the incorporation from outside the cell of
24 amino acids. In addition, the known insulin-like growth factors (1
25 and 2) have their cell receptors, and their agonism promotes the

1 tumor cell growth and, therefore, the tumor cell protein synthesis.
2 Insulin-like growth factors are very important in malignant growth,
3 and most tumor cells have a high level of insulin-like growth
4 factor-receptors. The cross-reactivity of insulin and insulin-like
5 growth factors and their receptors is known. In particular,
6 insulin promotes the protein synthesis mainly in tumor cells
7 because it is the agonist of its own receptor but also it is
8 cross-agonist of insulin-like growth factor-receptors highly
9 expressed in most malignant cells as it was referred.

10 It is noted, however, that other pharmaceutical compounds may
11 be suitable for use in the method of the present invention for
12 inducing protein synthesis in tumor cells, and that such
13 pharmaceutical compounds may be utilized either in combination with
14 or as a substitute for insulin. Among the other pharmaceutical
15 compounds known to exhibit insulin-like growth factors are,
16 somatotrophin, estrogens, androgens, just to name a few, however,
17 it is to be understood that any compound able to induce protein
18 synthesis in tumor cells may be suitable for use in the method of
19 the present invention.

20 In addition to inducing protein synthesis in a plurality of
21 tumor cells of the patient, the present invention comprises
22 generating chemical-induced gene mutations or epigenetic protein
23 modifications in the plurality of tumor cells by treating the
24 patient with DNA targeted chemotherapeutics, thereby resulting in
25 the generation of a plurality of proteins unrecognizable as

1 self-proteins by the patient's immune system which, as previously
2 indicated, are known as neo-antigens or tumor associated antigens
3 (TAA) .

4 Most of the compounds used in antitumoral chemotherapy include
5 agents structured to avoid DNA synthesis, which is required for
6 cell reproduction. In particular, these compounds may comprise
7 agents acting upon the structures of the DNA double helix that
8 avoids the kinetic or enzymatic activity in DNA duplication, for
9 example, cyclophosphamide, or enzymatic inhibitors acting upon
10 enzymes required for nucleotide antecessor synthesis, such as,
11 fluorouracil, or enzymes required for recovery of nucleotide
12 synthesis cofactors including such compounds as methotrexate.

13 All compounds used in antitumoral chemotherapy which interfere
14 with the normal DNA sequence can induce punctual or sectorial
15 mutations through the modification of polypeptide codification.
16 The significance of these mutations is that the immunologic non
17 self-recognizance by the patient's immune system is higher when
18 further mutagenic events are induced. In the present invention, at
19 least one, but preferably a plurality of such mutagenic drugs, or
20 DNA targeted chemotherapeutics, may be utilized which are
21 addressed with selectivity to the tumor cells. The selectivity of
22 tumor cells is determined by the high level of expressed
23 insulin-like growth factor-receptors, thereby allowing the DNA
24 targeted chemotherapeutics to reach the malignant cells through
25 the increased permeability and proliferative requirements induced

1 in these cells by the insulin.

2 In one alternate embodiment of the present invention, the
3 method comprises promoting mutations in tumor cells via
4 pharmacological agents and/or radiotherapeutical agents to produce
5 chemical-induced or physical-induced gene mutations or epigenetic
6 protein modifications, either in combination with or as a
7 substitute for the aforementioned DNA targeted chemotherapeutics.

8 At least one embodiment of the method of the present invention
9 further comprises at least temporarily preserving the plurality of
10 TAA within the plurality of cells of the patient. In one preferred
11 embodiment, the plurality of TAA is at least temporarily preserved
12 in the plurality of malignant tumor cells of the patient, by
13 promoting the synthesis of molecules which act as chaperones of
14 such intracellular peptides and proteins. The method of the
15 present invention thus further comprises the step of inducing the
16 synthesis of stress shock protein (SSP). The SSP is known as a
17 chaperone because it protects proteins, such as TAA, by generating
18 molecular complexes with them, thereby masking their presence to
19 the immune system of the patient, as well as other molecular
20 aggressors such as proteases. The induction of SSP may be
21 accomplished utilizing pharmacological agents which are similar,
22 and in at least one embodiment, identical to those utilized for
23 generating the plurality of TAA. Thus, in at least one
24 embodiment, the method of the present invention may accomplish the
25 dual objectives, generating TAA and inducing SSP, in a single step.

1 This is accomplished by the fact that the mechanisms involved in
2 TAA generation, share the property of inducing SSP synthesis.
3 Specifically, the present invention may employ the dual mechanisms
4 of insulin hypoglycemia and chemotherapeutical induced stress.

5 More in particular, cells which are submitted to heat or other
6 stress agents respond with the synthesis of a compound known as a
7 heat shock protein (HSP) or, more generally, stress shock protein
8 (SSP). The HSP or SSP have an inherent protective property for
9 other cellular proteins or peptides by forming molecular complexes
10 with them, at the risk, however, of the cellular proteins or
11 peptides being denatured by the HSP or SSP. As the HSP or SSP form
12 molecular complexes with these cellular proteins or peptides, the
13 HSP and SSP are also commonly known as chaperones molecules.

14 In the method of the present invention, the plurality of tumor
15 cells of the patient are exposed to such cellular stress via
16 hypoglycemia and antitumoral chemotherapeutics. As indicated
17 above, this exposure is performed simultaneously with the
18 generation of the plurality of TAA and, therefore, the chaperone
19 molecules induced by the method preserve and at least temporarily
20 store the plurality of TAA inside the plurality of tumor cells. In
21 at least one embodiment, the method may also comprise administering
22 indomethacin, cortisol derivatives, corticoid compounds, and other
23 pharmacological agents to the patient to initiate the generation of
24 SSP.

25 To elaborate further, when tumor cell stress is induced by

1 hypoglycemia through insulin treatment, it is noted that insulin,
2 which may also utilized by the method of the present invention for
3 inducing protein synthesis, in sufficient dosages produces
4 hypoglycemia, which induces SSP synthesis in cells subjected to
5 this glucose restrictive condition. Because malignant cells
6 normally require an elevated level of glycolysis to begin with,
7 hypoglycemia presents a particularly high level of risk for these
8 cells and, therefore, a particularly high level of stress, with a
9 subsequent high level of SSP or chaperone molecule synthesis which
10 may be utilized to at least temporarily preserve and store the
11 plurality of TAA in the plurality of malignant tumor cells.

12 In one further embodiment, the method of the present invention
13 may utilize other pharmacological or nutritional treatments to
14 compliment the insulin induced hypoglycemia, or as a substitute for
15 insulin to induce this condition in the patient so as to stress the
16 plurality of tumor cells, thereby accomplishing the objective of
17 generating SSP or chaperone molecules to at least temporarily
18 preserve and store the plurality of TAA in the tumor cells.

19 In one alternate embodiment, stress to the tumor cells may be
20 chemically induced by DNA targeted chemotherapeutics. In
21 particular, DNA targeted chemotherapeutics, similar to those
22 described above for use in mutagenic TAA generation, are also known
23 for inducing cell stress. Active metabolites of cyclophosphamide,
24 5-fluorouracil, and methotrexate, are just a few of the drugs used
25 in antitumoral chemotherapy which may also be employed by the

1 present invention to chemically stress the patient's tumor cells to
2 induce generation of SSP or chaperone molecules. As previously
3 indicated, the method of the present invention may employ these
4 drugs for simultaneously generating TAA and SSP.

5 The above method for generating SSP may be optimized when
6 conducted in conjunction with indomethacin, a drug which is very
7 well known for other uses in medicine, and has been recognized as
8 a promoter of SSP synthesis. Indomethacin is a positive modulator
9 of DNA binding to heat shock translational factor (hstf-1). This
10 factor, through DNA binding, starts and maintains SSP synthesis.

11 In one other alternate embodiment, the method of the present
12 invention may utilize other pharmacological or radiotherapeutical
13 agents to complement or as substitutes for the DNA targeted
14 chemotherapeutics described above to chemically or physically
15 stress tumor cells in the patient. Further, and as indicated
16 above, indomethacin, cortisol derivatives, corticoid compounds, as
17 well as other suitable pharmacologicals may be utilized in order to
18 initiate SSP generation, thereby, enhancing the preserving and
19 storing of the plurality of TAA in the plurality of tumor cells in
20 the patient.

21 The method of the present invention further comprises the step
22 of increasing the efficiency of the antitumoral immune response in
23 cancer patients. More in particular, the presentation of an
24 antigen to the immune system is facilitated by specific antigen
25 presenting cells (APC), mainly to the lymphocytes, such

1 presentation being necessary to elicit an immune response. At the
2 same time, however, the antitumoral efficiency of this response
3 requires avoiding the eliciting of an immune tolerance response to
4 the plurality of TAA.

5 To begin, activating a plurality of APC may be accomplished
6 via an adequate cytokine treatment, such as by administering a
7 granulocyte-macrophage colony stimulating factor (GM-CSF). Human
8 recombinant GM-CSF is known as an immune modulating cytokine that
9 increases the dendritic cell population promoting its maturation
10 and, as consequence, it amplifies the dendritic cell function of
11 antigen presentation in order to start the immune response. This
12 pharmacological property has been used to potentiate cancer
13 vaccines with different external immunogens. In the present
14 invention, and in particular, in an internal vaccine as previously
15 described, the GM-CSF activated plurality of APC encounter the
16 plurality of TAA which was previously preserved and stored in the
17 plurality of tumor cells of the patient's body, which have been
18 subsequently released into the patient's bloodstream via the
19 mechanisms of autoschizis and/or apoptosis, which are described in
20 further detail below. Additionally, the GM-CSF activated plurality
21 of APC may encounter the plurality of TAA contained in an external
22 vaccine comprising an autologous hemoderivative composition, as is
23 also discussed in greater detail below.

24 In one embodiment, other pharmacological or immunological
25 agents or biological response modifiers may be utilized to further

1 increase the antitumoral immune response of GM-CSF, either as a
2 complementary or substitutive methodological step.

3 In addition to increasing the encounters between the plurality
4 of APC and the plurality of TAA, the method of the present
5 invention further comprises breaking or inhibiting the immune
6 tolerance response via pharmacological treatment and, in one
7 preferred embodiment, by administering cyclophosphamide to the
8 patient in a specific chronological sequence with the generation of
9 the plurality of TAA.

10 Because the inventive method may be employed a plurality of
11 times over the course of the patient's entire treatment regimen, it
12 is necessary to minimize the immune tolerance response in the
13 patient typically elicited by the immune-stimulation that has been
14 described in cancer patients. Thus, the method of the present
15 invention utilizes low dosages of cyclophosphamide in a specific
16 chronological sequence with the antigenic stimulation to inhibit
17 the immune tolerance response in the patient, prior to
18 administration of both the internal vaccine and the external
19 vaccine. It is to be understood that while the method of the
20 present invention may utilize cyclophosphamide, it is not the
21 exclusive means for breaking or inhibiting the immune tolerance
22 response in the patient.

23 As indicated above, the present invention further comprises an
24 internal vaccine. More specifically, the method comprises
25 triggering the release of the plurality of TAA, which has been

1 preserved and stored in the plurality of tumor cells of the
2 patient, via a pharmacological tumor cell death that preserves the
3 immunogenicity of the plurality of TAA, or immunogenic cell death.

4 It is known that all chemotherapeutical treatments in oncology
5 kill tumor cells by apoptosis, but the immunogenicity of such tumor
6 cells is only preserved if these tumor cells are first exposed to
7 a cellular stress prior to being killed. Therefore, although known
8 antitumoral chemotherapy comprise a mechanism to induce tumor cell
9 death, a preferred embodiment of the present invention comprises a
10 mechanism for inducing pharmacological tumor cell death utilizing
11 a mechanism of cellular body fragmentation via high dosages of
12 ascorbic acid administered intravenously, which is known as
13 autoschizis, which may or may not be potentiated with menadione
14 administered to the patient simultaneously with the high dosages of
15 ascorbic acid. Thus, the method of the present invention triggers
16 the release of the plurality of TAA, preserved and stored in the
17 plurality of tumor cells of the patient, into the patient's body
18 via the various intracellular components through the interstitial
19 space including, but not limited to phagocytes, lymphatic vessels
20 and/or blood vessels, thereby allowing the plurality of TAA to
21 encounter the plurality of APC, and the patient's immune system,
22 thus initiating an autologous antitumoral immune response.

23 Following some controversy in the medical field with regard to
24 the effects of ascorbic acid on terminal cancer patients, it has
25 been demonstrated that ascorbic acid in high doses administered

1 intravenously is selectively cytotoxic for malignant cells and in
2 high doses in vitro, it induces tumor cell death through a modified
3 apoptosis mechanism known as autoschizis, as indicated above.
4 Specifically, autoschizis is a cell death with fragmentation of the
5 cell body and release of cell fragments and, more importantly with
6 respect to the method of the present invention, the cell contents
7 (i.e. the plurality of TAA) to the extracellular surroundings. As
8 previously noted, other mechanisms of cell death, such as classical
9 apoptosis, are only immunogenic if the cell has been exposed to the
10 cellular stress prior to death, such that the antigens inside the
11 cell are protected by chaperone compounds (e.g. SSP) induced by
12 said stress, otherwise, the antigen may also be destroyed in the
13 cell death process. The method of the present invention may
14 comprise, in addition to the mechanism of cell death via
15 autoschizis, a mechanism of cell death via chemotherapy-induced
16 apoptosis, however, the apoptosis cell deaths will be immunogenic,
17 because all of the tumor cells are exposed to cellular stress prior
18 to death under the method of the present invention.

19 Currently, research is being conducted to determine the
20 chemotherapeutic value of ascorbic acid in high dose administered
21 intravenously to cancer patients, including the use of ascorbic
22 acid potentiated by menadione. It is noted, however, that there
23 are no known applications of ascorbic acid autoschizis, or any
24 associated procedure, utilized to induce an antitumoral immune
25 response or to start an immunotherapy treatment regimen. In the

1 method of the present invention, an internal vaccine is obtained by
2 triggering the release of the plurality of TAA from the plurality
3 of malignant cells of the patient, at least partially into the
4 patient's bloodstream via autoschizis. As such, specimens of blood
5 containing at least some of the released plurality of TAA may
6 subsequently be utilized to prepare an external vaccine, as
7 discussed below.

8 At least one alternate embodiment of the method of the present
9 invention further comprises the use of menadione or another
10 pharmacological agent to potentiate the mechanism of autoschizis
11 and/or the use of chemotherapy and/or the use of radiotherapy in
12 combination with or as a substitute for the intravenous
13 administration of ascorbic acid to induce tumor cell death and the
14 subsequent, immunogenic release of the plurality of TAA into the
15 patient's system.

16 The method of the present invention further comprises
17 administering an external vaccine to cancer patients, the external
18 vaccine preferably comprising an autologous hemoderivative
19 composition prepared from an autologous blood specimen containing
20 at least some of the plurality of TAA released from tumor cells.

21 The present invention further provides a method for producing
22 such an external vaccine. In particular, at least some of the
23 plurality of TAA released into the patient's blood as molecular
24 chaperone protected complexes as a result of the internal vaccine
25 are distributed in blood cells and blood plasma where they may

1 become associated by external adhesion or by phagocytosis. When
2 such a blood specimen is exposed to a hypotonic and hypothermic
3 shock, essentially all of the plurality of TAA-chaperone complexes
4 are released from the blood cells, into a supernatant. Afterwards,
5 the supernatant may be exposed to thermal fractioning, such as by
6 heating to approximately 100 degrees centigrade for approximately
7 between 8 to 10 minutes. Under these conditions, the TAA-chaperone
8 complexes are opened, and the plurality of TAA become free. In
9 addition, under these conditions, a majority of the enzymatic and
10 toxic properties of other molecules contained in the preparation
11 are destroyed, but the immunogenic properties of the plurality of
12 TAA is preserved. The method of the present invention further
13 provides for filtration of the subsequent solution thereby
14 resulting in the external vaccine comprising an autologous
15 hemoderivative composition. The method of the present invention
16 further provides for the inoculation of the patient, such as via
17 subcutaneous injection, which is known for its efficiency to
18 promote encounters between antigens and APC in other vaccination
19 procedures.

20 A particular and significant advantage of preparing the
21 external vaccine utilizing the method of the present invention is
22 that the entire method requires minimal laboratory facilities,
23 thereby providing a simple, safe, and economical method to prepare
24 a vaccine, relative to those prepared in highly complex facilities
25 where autologous biological specimens must be transported.

1 A more detailed description of the method for preparing an
2 external vaccine comprising an autologous hemoderivative
3 composition is as follows. The method of the present invention
4 provides for extracting a blood specimen of approximately 20
5 milliliters from a femoral artery of the patient into a first
6 syringe pre-filled with approximately 5,000 international units
7 (I.U.) of heparin having a concentration in a range of between
8 approximately 250 to 300 I.U. per milliliter. The blood specimen
9 solution is allowed to sediment or settle in vertical position at
10 a temperature of approximately 37 degrees centigrade. After
11 approximately one hour, an aliquot of a supernatant of white cell
12 rich blood plasma is separated from the blood specimen solution
13 into a second syringe containing between approximately 3 to 4 parts
14 of distilled water per part of the plasma-cell layer forming a
15 plasma-cell solution and, thereby, inducing a hypotonic cytolysis.
16 The method of the present invention further provides that the
17 plasma-cell solution be stored at approximately minus twenty
18 degrees centigrade for a period of approximately 24 hours, after
19 which, the plasma-cell solution is warmed up to approximately 37
20 degrees centigrade in order to complete the hypotonic-hypothermic
21 cytolysis process.

22 The resultant plasma-cell solution may be filtered through a
23 glass wood membrane or optionally it is centrifuged at 2000 G, in
24 order to clear the solution and remove gross precipitates. In yet
25 another embodiment, the resultant plasma-cell solution may be

1 sonicated to clear the solution and remove gross precipitates. The
2 resultant plasma-cell solution is then subjected to further thermal
3 treatment. More in particular, the method of the present invention
4 permits utilization of any one of a plurality of thermal treatments
5 in order to obtain different immunogens. In one preferred
6 embodiment, the plasma-cell solution is heated to approximately 100
7 degrees centigrade for approximately between 8 to 10 minutes. The
8 method also provides for allowing the solution to return to room
9 temperature, approximately 25 degrees centigrade, until temperature
10 equilibrium is reached. Finally, the resultant plasma-cell
11 fraction may also be either filtered through glass wool membrane,
12 centrifuged at 2000 G, or sonicated, followed by filtration through
13 cellulose membranes ranging from between approximately 0.20 to 0.45
14 μm diameter.

15 While the method of the present invention for preparing an
16 external vaccine presented above comprises one preferred
17 embodiment, it is understood that alternative embodiments may be
18 utilized to prepare an external vaccine comprising an autologous
19 hemoderivative composition through modification of the methods for
20 blood extraction, sedimentation, and/or specific temperatures and
21 durations for thermal fractionation. In addition, it is understood
22 that while a preferred embodiment of the present invention
23 comprises subcutaneous inoculation of the patient with the external
24 vaccine, inoculation via other mechanisms including, by way of
25 example only, intradermal, intravenous, and/or intramuscular

1 vaccination are encompassed in the method of the present invention
2 to elicit an efficient antitumoral immune response or an
3 antitumoral biological response targeted to tumor cells, tumor
4 stroma patient's immune system and/or molecular mediators of the
5 host biological response against cancer disease.

6 Thus, from the foregoing, it is readily seen that the method
7 of the present invention allows one to elicit an antitumoral immune
8 response in a cancer patient which may be addressed against his or
9 her own specific tumor. In addition, the method provides for the
10 pharmacological management of a patient's own cancer cell's antigen
11 library to increase a malignant tumor's antigenicity. Also, the
12 method comprises releasing an internal autologous vaccine from a
13 patient's own tumor(s), specific antigens eliciting an antitumoral
14 immune response against the patient's remaining malignant cancer
15 cells. A further aspect of the present invention is a method of
16 preparing and providing an external autologous vaccine comprising
17 a hemoderivative composition obtained at least in part from
18 inducing the generation and subsequent release into a patient's
19 bloodstream of tumor specific TAA. Yet another aspect of the
20 present invention is a method to enhance the antitumoral immune
21 response in a cancer patient by activating an APC population
22 induced by cytokine treatment and inhibiting tolerance immune
23 response in the patient. The present invention further provides a
24 method for an immunologically assessing an immune response elicited
25 by an autologous vaccine of a cancer patient via an intradermal

1 test, as well as a method for assessing an antitumoral response
2 elicited by an autologous vaccine of the cancer patient. Most
3 importantly, the method of the present invention provides an
4 innovative and alternate technique for eliciting an antitumoral
5 immune response in a cancer patient in the event that surgery,
6 chemotherapy, radiotherapy, and/or other cancer treatment regimens
7 fail.

8 These and other objects, features and advantages of the
9 present invention will become more clear when the figures as well
10 as the detailed description are taken into consideration.

11

12 BRIEF DESCRIPTION OF THE DRAWINGS

13 For a fuller understanding of the nature of the present
14 invention, reference should be had to the following detailed
15 description taken in connection with the accompanying figures in
16 which:

17 Figure 1 is a schematic view of one preferred embodiment of
18 the inventive method to elicit an effective autologous antitumoral
19 immune response in a patient.

20 Figure 2 is a schematic of the embodiment of Figure 1 further
21 illustrating one preferred embodiment for generating and preserving
22 a plurality of tumor associated antigens (TAA) in a plurality of
23 cells in the patient.

24 Figure 3 is a schematic of the embodiment of Figure 1 further
25 illustrating one preferred embodiment for activating a plurality of

1 antigen presenting cells (APC) in the patient.

2 Figure 4 is a schematic of the embodiment of Figure 1 further
3 illustrating one preferred embodiment for inhibiting an immune
4 tolerance response for the TAA in the patient.

5 Figure 5 is a schematic of the embodiment of Figure 1 further
6 illustrating one preferred embodiment for triggering an internal
7 vaccine in the patient.

8 Figure 6 is a schematic of the embodiment of Figure 1 further
9 illustrating one preferred embodiment for preparing and providing
10 an external vaccine to the patient.

11

12 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

13 While this invention is susceptible of embodiment in many
14 different forms, there is shown in the figures and will herein be
15 described in detail at least one specific embodiment, with the
16 understanding that the present disclosure is to be considered as an
17 exemplification of the principles of the invention and is not
18 intended to limit the invention to the embodiment illustrated.

19 As indicated above, the present invention is directed in
20 general to a new, improved, and innovative active specific
21 immunotherapy technique. More in particular, the present invention
22 is directed to a method and a composition to elicit an effective
23 antitumoral immune response in a patient, specific to his or her
24 own tumor antigens (i.e. an autologous antitumoral immune
25 response). Thus, more specifically, the present invention is

1 directed to a method and composition to elicit an effective
2 autologous antitumoral immune response in a cancer patient which
3 comprises generating, preserving, and storing specific tumor
4 associated antigens, and eliciting the autologous antitumoral
5 immune response, at least in part, through a combination of dual
6 vaccines. In addition, the present invention provides enhancement
7 of the antitumoral immune response resulting from an internal
8 vaccine and an external vaccine by activating antigen presenting
9 cells, as well as by inhibiting a tolerance immune response in
10 cancer patients. The present invention further provides a method
11 for preparing a hemoderivative composition, and, more in
12 particular, an autologous hemoderivative composition, for
13 utilization in the inventive method as an external vaccine. Figure
14 1 presents a schematic view of one preferred embodiment of the
15 method of the present invention. More in particular, Figure 1
16 illustrates one preferred embodiment of one complete treatment
17 cycle of the method of the present invention. The method of the
18 present invention may comprise completing a single treatment cycle,
19 however, at least one embodiment of the present invention includes
20 completing a plurality of treatment cycles.

21 To begin, the method of the present invention provides for
22 generating a plurality of tumor associated antigens (TAA) in a
23 plurality of cells of the patient. As indicated above, mutated
24 genes can generate a plurality of TAA by their direct expression or
25 by the promotion of intracellular conditions eliciting epigenetic

1 normal protein transformation. In order to generate the production
2 of a plurality of TAA in tumor cells, it is necessary to increase
3 in these cells their protein synthesis and mutation frequency.

4 Thus, at least one embodiment of the method of the present
5 invention further comprises inducing protein synthesis in a
6 plurality of tumor cells by treating the patient with a suitable
7 pharmaceutical compound in order to activate the growth
8 factor-receptors, such as are typically highly expressed in most
9 malignant cells. One pharmaceutical compound which is suitable for
10 this purpose is insulin, due to the insulin-like growth factor-
11 receptors which are highly expressed in many malignant cells. In
12 particular, insulin promotes the protein synthesis mainly in tumor
13 cells because it is the agonist of its own receptor but also it is
14 cross-agonist of insulin-like growth factor-receptors highly
15 expressed in most malignant cells as it was referred.

16 It is noted, however, that other pharmaceutical compounds may
17 be suitable for use in the method of the present invention for
18 inducing protein synthesis in tumor cells, and that such
19 pharmaceutical compounds may be utilized either in combination with
20 or as a substitute for insulin. Among the other pharmaceutical
21 compounds known to exhibit insulin-like growth factors are,
22 somatotrophin, estrogens, androgens, just to name a few, however,
23 it is to be understood that any compound able to induce protein
24 synthesis in tumor cells may be suitable for use in an embodiment
25 of the method of the present invention.

1 In addition to inducing protein synthesis in the plurality of
2 cells of the patient, the present invention comprises generating
3 chemical-induced gene mutations or epigenetic protein modifications
4 in the plurality of tumor cells by treating the patient with DNA
5 targeted chemotherapeutics, thereby resulting in the generation
6 of a plurality of proteins unrecognizable as self-proteins by the
7 patient's immune system which, as previously indicated, are known
8 as neo-antigens or tumor associated antigens (TAA).

9 Many of the compounds used in antitumoral chemotherapy include
10 agents structured to avoid DNA synthesis, which is required for
11 cell reproduction. In particular, DNA targeted chemotherapeutics
12 comprise agents which act upon the structures of the DNA double
13 helix that avoid the kinetic or enzymatic activity in DNA
14 duplication and include, but are not limited to, cyclophosphamide,
15 or enzymatic inhibitors acting upon enzymes required for nucleotide
16 antecessor synthesis, such as, fluorouracil, or enzymes required
17 for recovery of nucleotide synthesis cofactors including such
18 compounds as methotrexate. One embodiment of the present invention
19 may comprise administering at least one of these DNA targeted
20 chemotherapeutics to the patient during a preparatory treatment
21 phase. In one preferred embodiment, as illustrated in Figure 2,
22 the method of the present invention comprises administering a
23 plurality of DNA targeted chemotherapeutics to the patient during
24 the preparatory treatment phase.

25 Thus, the method of the present invention provides for

1 administering one or more compounds to the patient during a
2 preparatory phase of the treatment cycle for generating a plurality
3 of TAA. The compounds selected to be administered to the patient
4 are among those known to induce protein synthesis, such as insulin,
5 as well as to generate production of a plurality of tumor
6 associated antigens (TAA) in a plurality of the cells, and in one
7 preferred embodiment, the malignant tumor cells of the patient.

8 As illustrated in Figure 2, one preferred embodiment of the
9 method of the present invention comprises administering insulin to
10 the patient each day of the preparatory treatment phase or, more
11 specifically, days one through four of the treatment cycle, at a
12 daily dosage of approximately 0.3 international units (I.U.) per
13 kilogram of body weight. In addition, Figure 2 also illustrates
14 that in a preferred embodiment, a plurality of DNA targeted
15 chemotherapeutics are administered to the patient during the
16 preparatory treatment phase (e.g. days one through four of the
17 treatment cycle). Specifically, one preferred embodiment of the
18 method of the present invention comprises administering
19 cyclophosphamide, at a daily dosage in a range of between
20 approximately 100 to 200 milligrams, methotrexate, at a daily
21 dosage in a range of between approximately 2.5 to 12.5 milligrams,
22 and fluorouracil, at a daily dosage in a range of between
23 approximately 125 to 250 milligrams, to the patient each day of the
24 preparatory treatment phase. In at least one embodiment of the
25 present invention, the preparatory phase comprises days one through

1 five of the treatment cycle.

2 At least one embodiment of the method of the present invention
3 further comprises at least temporarily preserving the plurality of
4 TAA within the plurality of cells of the patient. In one preferred
5 embodiment, the plurality of TAA is at least temporarily preserved
6 in the plurality of malignant tumor cells of the patient, by
7 promoting the synthesis of molecules which act as chaperones of
8 such intracellular peptides and proteins. The method of the
9 present invention thus further comprises the step of inducing the
10 synthesis of stress shock protein (SSP). The SSP is known as a
11 chaperone because it protects proteins, such as TAA, by generating
12 molecular complexes with them, thereby masking their presence to
13 the immune system of the patient, as well as other molecular
14 aggressors such as proteases. The induction of SSP may be
15 accomplished utilizing pharmacological agents which are similar,
16 and in at least one embodiment, identical to those utilized for
17 generating the plurality of TAA. Thus, in at least one
18 embodiment, the method of the present invention may accomplish the
19 dual objectives of generating TAA and inducing SSP in a single
20 step. This is accomplished by the fact that the mechanisms
21 involved in TAA generation, are similar to those for inducing the
22 synthesis of SSP. Specifically, the present invention may employ
23 the dual mechanisms of insulin hypoglycemia and chemotherapeutical
24 induced stress.

25 Thus, in the method of the present invention, the plurality of

1 tumor cells of the patient are exposed to cellular stress via
2 hypoglycemia and antitumoral chemotherapeutics. As indicated
3 above, this exposure is performed simultaneously with the
4 generation of the plurality of TAA and, therefore, the chaperone
5 molecules induced by the method preserve and at least temporarily
6 store the plurality of TAA inside the plurality of tumor cells. In
7 at least one embodiment, the method may further comprise
8 administering indomethacin, cortisol derivatives, corticoid
9 compounds, and other pharmacological agents to the patient to
10 initiate the generation of the plurality of SSP.

11 As such, at least one embodiment of the present invention
12 further comprises preserving the plurality of TAA by inducing the
13 synthesis of a plurality of SSP. More in particular, the method of
14 the present invention may include inducing the synthesis of the SSP
15 comprises by administering indomethacin to the patient. In one
16 alternate embodiment, the method of the present invention may
17 include inducing the synthesis of the SSP by administering a
18 corticoid compound to the patient.

19 Also, as indicated above, the method of the present invention
20 further comprises storing the TAA in the plurality of cells of the
21 patient by inducing the synthesis of a plurality of stress shock
22 proteins (SSP). Once again, the method may comprise inducing the
23 synthesis of the SSP comprises by administering indomethacin to the
24 patient. In one alternate embodiment, the method of the present
25 invention may include inducing the synthesis of the SSP by

1 administering a corticoid compound to the patient.

2 The method of the present invention also comprises breaking or
3 inhibiting the immune tolerance response relative to the TAA
4 generated in the cells of the patient to enhance the antitumoral
5 immune response. In at least one embodiment, inhibiting the immune
6 tolerance response is accomplished via pharmacological treatment
7 and, in one preferred embodiment, by administering cyclophosphamide
8 to the patient in a specific chronological sequence with the
9 generation of the plurality of TAA.

10 Because the inventive method may comprise completing a
11 plurality of treatment cycles over the course of the patient's
12 entire treatment regimen, it becomes necessary to minimize the
13 immune tolerance response in the patient typically elicited by the
14 immune-stimulation that has been described in cancer patients.
15 Thus, in at least one embodiment, the method of the present
16 invention utilizes low dosages of cyclophosphamide to inhibit the
17 immune tolerance response in the patient, prior to administration
18 of both the internal vaccine and the external vaccine. It is to be
19 understood that while the method of the present invention may
20 utilize cyclophosphamide, it is not the exclusive means for
21 breaking or inhibiting the immune tolerance response in the patient
22 encompassed by and which may be utilized in conjunction with the
23 method of the present invention.

24 In particular, one embodiment of the method of the present
25 invention comprises administering cyclophosphamide to the patient

1 during a first intermediate phase of the treatment cycle. More
2 specifically, and as illustrated in Figure 4, one preferred
3 embodiment of the method of the present invention comprises
4 administering cyclophosphamide at a dosage of approximately 300
5 milligrams per square meter of surface area of the patient's body,
6 on day five of the treatment cycle.

7 The method of the present invention further comprises
8 activating a plurality of antigen presenting cells (APC) in the
9 patient to further enhance the antitumoral immune response. More
10 in particular, the presentation of an antigen to the immune system
11 is facilitated by specific APC, mainly to the lymphocytes, and is
12 necessary to elicit an immune response. At the same time, however,
13 the antitumoral efficiency of this response requires avoiding the
14 eliciting of an immune tolerance response to the plurality of TAA.

15 In at least one embodiment, activating a plurality of APC may
16 be accomplished via an adequate cytokine treatment, such as, for
17 example, administering a granulocyte-macrophage colony stimulating
18 factor (GM-CSF). Human recombinant GM-CSF is known as an immune
19 modulating cytokine that increases the dendritic cell population
20 promoting its maturation and, as consequence, it amplifies the
21 dendritic cell function of antigen presentation in order to start
22 the immune response. In the present invention, and in particular,
23 in conjunction with an internal vaccine as previously described,
24 the GM-CSF activated plurality of APC encounter the plurality of
25 TAA released into the patient's bloodstream via the mechanisms of

1 autoschizis and/or apoptosis, as previously described in detail.
2 Additionally, the GM-CSF activated plurality of APC may encounter
3 the plurality of TAA contained in an external vaccine comprising an
4 autologous hemoderivative composition, as also discussed in further
5 detail below. It is understood to be within the scope of the
6 method of the present invention to administer alternate
7 pharmacological or immunological agents or biological response
8 modifiers to either increase the antitumoral immune response of
9 GM-CSF, or as a substitute for GM-CSF.

10 In one preferred embodiment, the method of the present
11 invention comprises activating a plurality of antigen presenting
12 cells (APC), to further enhance the antitumoral immune response in
13 the patient, by administering a cytokine to the patient during a
14 primary treatment phase. In at least one embodiment, the method of
15 the present invention includes administering the cytokine
16 comprising granulocyte-macrophage colony stimulating factor (GM-
17 CSF) to the patient. As illustrated in Figure 3, one preferred
18 embodiment of the method of the present invention comprises
19 administering GM-CSF to the patient on each day of the primary
20 treatment phase at a daily dosage in a range of between
21 approximately 150 to 250 micrograms. In at least one embodiment,
22 the primary treatment phase of the method comprises day eight
23 through twelve of the treatment cycle.

24 As discussed above in some detail, the method of the present
25 invention further comprises the new and innovative feature of

1 triggering an internal vaccine in the patient. As disclosed
2 herein, the internal vaccine comprises the release of the TAA
3 previously generated, preserved, and stored in the plurality of
4 tumor cells of the patient's body, via a tumor cell death that
5 preserves the immunogenicity of the TAA, known as an immunogenic
6 cell death.

7 In particular, the method of the present invention provides
8 for triggering the release of the plurality of TAA into the
9 patient's body via the various intracellular components through the
10 interstitial space including, but not limited to phagocytes,
11 lymphatic vessels and/or blood vessels, thereby allowing the
12 plurality of TAA to encounter the plurality of APC, and the
13 patient's immune system, thereby initiating an autologous
14 antitumoral immune response. In at least one embodiment, the
15 method of the present invention includes administering ascorbic
16 acid to the patient during the primary treatment phase to induce
17 immunogenic cell death through a modified apoptosis mechanism known
18 as autoschizis. In one preferred embodiment, the internal vaccine
19 is triggered via administering the ascorbic acid to the patient
20 intravenously, for example, in a lactate-ringer solution.

21 As illustrated in Figure 5, a preferred embodiment of the
22 present invention comprises triggering the internal vaccine by
23 inducing autoschizis by administering ascorbic acid to the patient
24 during each day of the primary treatment phase. More specifically,
25 the preferred embodiment of the method includes administering the

1 ascorbic acid to the patient each day of the primary treatment
2 phase at a daily dosage of approximately 25 grams in approximately
3 250 milliliters of a lactate-ringer solution. As noted above, the
4 ascorbic acid is preferable administered intravenously. As also
5 noted above, in at least one embodiment of the method of the
6 present invention, the primary treatment phase includes days eight
7 through twelve of the treatment cycle.

8 Alternatively, the method may comprise, either in lieu of or
9 in addition to the mechanism of cell death via autoschizis, a
10 mechanism of cell death via chemotherapy-induced apoptosis,
11 however, the apoptosis cell death induced by the method of the
12 present invention will be an immunogenic cell death, because all of
13 the tumor cells are exposed to cellular stress prior to death.

14 At least one embodiment of the method of the present invention
15 further comprises the administration of menadione or another
16 pharmacological agent to potentiate the mechanism of autoschizis
17 and/or the use of chemotherapy and/or the use of radiotherapy in
18 combination with or as a substitute for the intravenous
19 administration of ascorbic acid to induce the immunogenic cell
20 death and the subsequent, immunogenic release of the plurality of
21 TAA into the patient's system.

22 As Figure 5 further illustrates, an alternate embodiment of
23 the method of the present invention further comprises administering
24 the menadione to the patient during the primary treatment phase
25 (e.g. days eight through twelve of the treatment cycle).

1 Specifically, the method of the present invention includes
2 administering the menadione to the patient each day of the primary
3 treatment phase at a daily dosage of approximately 250 milligrams.
4 In one preferred embodiment, the method comprises administering the
5 menadione to the patient intravenously, however, in at least one
6 alternate embodiment, the menadione may be administered orally.

7 The method of the present invention further comprises
8 providing an external vaccine to cancer patients, the external
9 vaccine comprising a hemoderivative composition, and preferably, an
10 autologous hemoderivative composition prepared from a blood
11 specimen from the patient and, thus, containing at least some of
12 the plurality of TAA released from tumor cells. In particular, the
13 method of the present invention includes administering an external
14 vaccine to the patient during a secondary phase of the treatment
15 cycle. As illustrated in Figure 6, in one preferred embodiment,
16 the external vaccine is administered to the patient on each of days
17 fifteen, seventeen, nineteen, twenty-two, twenty-four, and twenty-
18 six of the treatment cycle. It is well understood that numerous
19 variations of this preferred schedule for administering the
20 external vaccine during the secondary treatment phase are
21 encompassed by the scope of the method of the present invention.

22 In at least one embodiment, the external vaccine is
23 administered subcutaneously, however, it is also understood to be
24 within the scope of the present invention to include administering
25 the external vaccine to the patient via alternate inoculation

1 mechanisms, including, but not limited to, intradermal and
2 intramuscular inoculations.

3 One alternate embodiment of the present invention further
4 comprises administering cyclophosphamide to the patient each day of
5 a second intermediate treatment phase at a daily dosage of
6 approximately 300 milligrams per square meter of surface area of
7 the patient's body. In at least one embodiment, the second
8 intermediate treatment phase comprises day thirteen of the
9 treatment cycle.

10 The present invention further comprises a method for preparing
11 the autologous hemoderivative composition, as illustrated
12 schematically in Figure 6, for use in eliciting an effective
13 antitumoral immune response in a patient, such as may be utilized
14 in the method described herein. In one preferred embodiment, the
15 method for preparing the autologous hemoderivative composition
16 includes extracting a blood specimen of approximately 20
17 milliliters from a femoral artery of the patient into a first
18 syringe pre-filled with approximately 5,000 international units
19 (I.U.) of heparin having a concentration in a range of between
20 approximately 250 to 300 I.U. per milliliter. The blood specimen
21 solution is allowed to settle while maintained in vertical position
22 at a temperature of approximately 37 degrees centigrade. After
23 approximately one hour, an aliquot of a supernatant of white cell
24 rich blood plasma is separated from the blood specimen solution
25 into a second syringe containing between approximately 3 to 4 parts

1 of distilled water per part of the plasma-cell layer thereby
2 forming a plasma-cell solution and, inducing a hypotonic cytolysis.
3 The method of the present invention further provides that the
4 plasma-cell solution be stored at approximately minus twenty
5 degrees centigrade for a period of approximately 24 hours, after
6 which, the plasma-cell solution is warmed up to approximately 37
7 degrees centigrade in order to complete a hypotonic-hypothermic
8 cytolysis process.

9 The resultant plasma-cell solution may be filtered through a
10 glass wood membrane or optionally it may be centrifuged at 2000 G,
11 in order to clear the solution and remove gross precipitates. In
12 yet another embodiment, the resultant plasma-cell solution may be
13 sonicated to clear the solution and remove the precipitates. The
14 resultant plasma-cell solution is then subjected to further thermal
15 treatment. More in particular, the method of the present invention
16 permits utilization of any one of a plurality of thermal treatments
17 in order to obtain different immunogens. In one preferred
18 embodiment, the plasma-cell solution is heated to approximately 100
19 degrees centigrade for approximately between 8 to 10 minutes,
20 thereby forming a plasma-cell fraction. The method also provides
21 for allowing the solution to return to room temperature,
22 approximately 25 degrees centigrade, until temperature equilibrium
23 is reached. Finally, the resultant plasma-cell fraction may also
24 be either filtered through glass wool membrane, centrifuged at 2000
25 G, or sonicated, followed by filtration through cellulose membranes

1 ranging from between approximately 0.20 to 0.45 μm diameter.

2 While the method of the present invention for preparing an
3 external vaccine presented above comprises one preferred
4 embodiment, it is understood that alternative embodiments may be
5 utilized to prepare an external vaccine comprising an autologous
6 hemoderivative composition through modification of the methods for
7 blood extraction, sedimentation, and/or specific temperatures and
8 durations for thermal fractionation. In addition, it is understood
9 that while a preferred embodiment of the present invention
10 comprises subcutaneous inoculation of the patient with the external
11 vaccine, inoculation via other mechanisms including, by way of
12 example only, intradermal, intravenous, and/or intramuscular
13 inoculation are encompassed in the method of the present invention
14 to elicit an efficient antitumoral immune response or an
15 antitumoral biological response targeted to tumor cells, tumor
16 stroma patient's immune system and/or molecular mediators of the
17 host biological response against cancer disease.

18 The present invention is also directed towards an autologous
19 hemoderivative composition, which may be utilized as an external
20 vaccine in the method to elicit an effective antitumoral immune
21 response disclosed herein. In one preferred embodiment, the
22 autologous hemoderivative composition comprises a plasma-cell
23 solution which has been cooled to approximately minus twenty
24 degrees centigrade for approximately 24 hours. The cooled plasma-
25 cell solution may subsequently be heated to approximately 100

1 degrees centigrade and fractioned for between approximately 8 to 10
2 minutes thereby forming a plasma-cell fraction, after which, the
3 plasma-cell fraction may be filtered upon cooling, and readied to
4 provide to the patient as an external vaccine.

5 The plasma-cell solution of the autologous hemoderivative
6 composition may be at least partially defined by a supernatant
7 plasma-cell layer which is separated from a blood specimen solution
8 and a quantity of distilled water, typically, 3 to 4 parts of
9 distilled water per part of the blood specimen solution.

10 In addition, the blood specimen solution may comprise a blood
11 specimen extracted from a patient, preferably, from femoral artery
12 of the patient, into a solution comprising, in at least one
13 embodiment, approximately 5,000 international units (I.U.) of
14 heparin at a concentration in a range of between approximately 250
15 to 300 I.U. per milliliter.

16 Since many modifications, variations and changes in detail can
17 be made to the described preferred embodiment of the invention, it
18 is intended that all matters in the foregoing description and
19 illustrated in the accompanying figures be interpreted as
20 illustrative and not in a limiting sense. Thus, the scope of the
21 invention should be determined by the appended claims and their
22 legal equivalents.

23 Now that the invention has been described,

24